Abstract 46

STREAMLINING MITOCHONDRIAL DNA SEQUENCING OF REFERENCE SAMPLES

Rhonda K. Roby[1,2], MPH, MS; Phillip Williamson[1], PhD; Melody Josserand[1], MS; John V. Planz[1], PhD; José A. Lorente[2], MD, PhD and Arthur J. Eisenberg[1], PhD
[1]University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107 USA
[2]Laboratory of Genetic Information, Department of Legal Medicine, University of Granada, Avenida de Madrid 11, Granada 18012 Spain

There are over 100,000 active missing persons' cases being worked each day with many of these individuals having disappeared under suspicious circumstances. It is estimated that over 14,000 sets of remains currently exist in medical examiner, coroner and law enforcement facilities in the United States. This does not include the countless number of remains that have been buried in pauper's graves or cremated without the retention of a sample for future identification purposes. Alarmingly, a significant percentage of these remains are associated with homicides cases.

The University of North Texas Center for Human Identification has pioneered a multi-faceted approach to the identification of unknown human remains for missing person and cold case investigations. It has integrated forensic anthropology and odontology with the most current DNA testing methods to provide a proactive outreach program available to criminal investigators nationwide to help identify missing persons and skeletal remains with the collection of family reference samples. As of May, 2007, 43 states have submitted approximately 1,000 human remains and 2,000 reference samples, which have resulted in over 100 identifications.

In order to address the increasing numbers of reference samples and in order to help reduce costs in this massive undertaking, a number of steps have been put into practice. Previously, we reported on the high throughput extraction process implemented for this program (Plopper, FJ *et al.*, "High throughput processing of family reference samples for missing persons programs: The use of robotics in extraction and amplification setup for STR and mtDNA analysis," Genetic Identity Conference Proceedings 17th International Symposium on Human Identification – 2006, Nashville, Tennessee).

We have executed several steps for the high throughput sequencing of family reference specimens while maintaining the quality of previous procedures. These steps include the adoption of BigDye® Terminator v.1.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA) to replace of dRhodamine Terminator Cycle Sequencing Kits (Applied Biosystems); reduction of dye chemistry kit consumption by using a sequence enhancing and dilution buffer; and a simple bead purification method to remove unincorporated BigDye® terminators. All of these steps can easily be implemented into robotic workflow. Lastly, Sequence Scanner Software v.1.0 (Applied Biosystems), a tool to quickly assess sequence quality, will be presented to demonstrate its utility in evaluating sequence data.

The poster will focus on: 1) the process developed to reduce the time and costs associated with mtDNA processing of family reference samples; 2) a comparison of previous procedures with the results from the more automated process; and 3) the overall analysis of a software program for quality assurance and quality control processes.